

TITLE OF THE INVENTION:

PRODUCTION PROCESS OF GLUCOSE-1-PHOSPHATE

BACKGROUND OF THE INVENTION

5 Field of the Invention:

The present invention relates to a process for producing glucose-1-phosphate making use of microorganisms.

Description of the Background Art:

10 Glucose-1-phosphate (hereafter abbreviated as "G-1-P") is useful as a substrate for syntheses of drugs and saccharides.

G-1-P is mainly obtained by phosphorolysis of starch or dextrin with maltodextrin phospholyase (MDPase), and a process making use of MDPase derived from potato (Japanese Patent Publication No. 95492/1994) and the so-called enzymatic process using MDPase derived from microorganisms have been reported to date.

20 As examples of the enzymatic process using MDPase derived from microorganisms, have been reported a process using an enzyme derived from *Escherichia coli* (Enzyme Microb. Technol. 17, 140-146 (1995) and a process using an enzyme derived from  
25 *Corynebacterium callunae* (J. Carbohydrate Chem., 14, 1017-1028 (1995)). More recently, processes for producing G-1-P making use of heat-stable MDPase

derived from medium thermophilic bacteria and high thermophilic bacteria such as *Bacillus stearothermophilus* (Japanese Patent Application Laid-Open No. 14580/1998) and *Thermus caldophilus* (J. Industrial Microbiol., 24, 89-93 (2000)) have been reported.

However, such enzymatic processes using the enzyme itself require complicated steps such as a step of extracting an enzyme from plants or bacteria and preparation of an immobilized enzyme, and it has hence been desirable to develop a simpler process for producing G-1-P.

#### SUMMARY OF THE INVENTION

It is an object of the present invention to provide a process for producing G-1-P by which a mass of G-1-P can be provided without using complicated steps.

The present inventors have carried out various investigations as to bacteria producing a mass of G-1-P in a medium by culture. As a result, it has been found that when bacteria of the genus *Corynebacterium* are cultured under conditions of the presence of a saccharide and a high concentration of phosphoric acid or a derivative thereof, a high concentration of G-1-P can be produced directly in a medium to produce a mass of G-1-P.

According to the present invention, there is thus provided a process for producing glucose-1-phosphate, comprising the steps of culturing bacteria of the genus *Corynebacterium* in a medium containing a saccharide and at least 1 mM of phosphoric acid or a derivative or salt thereof, and collecting glucose-1-phosphate produced and accumulated in the medium.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The bacteria of the genus *Corynebacterium* used in the present invention are not particularly limited so far as they belong to the genus *Corynebacterium* and produce G-1-P in a medium in the presence of a saccharide and a fixed concentration of phosphoric acid or a derivative or salt thereof, and examples thereof include *Corynebacterium callunae*, *Corynebacterium glutamicum*, *Corynebacterium vitaeruminis* and *Corynebacterium pilosum*. Among these, *Corynebacterium callunae*, *Corynebacterium glutamicum* and *Corynebacterium vitaeruminis* are preferred, with *Corynebacterium callunae* IFO 15359, *Corynebacterium glutamicum* JCM 1321 and *Corynebacterium vitaeruminis* JCM 1323 strains being particularly preferred.

Preferable examples of the saccharide added to the medium include monosaccharides, disaccharides, oligosaccharides and polysaccharides containing

glucose as a constitutive saccharide, and  $\alpha$ -1,4-glucane-containing saccharides, for example, starch, amylose, dextrin, maltose, maltooligosaccharides, amylopectin and glycogen are mentioned as more  
5 preferable examples. Among these, cheap starch, dextrin and maltooligosaccharides are particularly preferred.

Such saccharides may be used either singly or in any combination thereof.

10 Examples of the derivative or salt of phosphoric acid to be added to the medium include metaphosphoric acid, tripolyphosphoric acid, polyphosphoric acid, diphosphoric acid, polymetaphosphoric acid, phosphates and salts of these derivatives. As the  
15 salts, are preferred sodium and potassium salts. Examples of particularly preferred phosphates include monopotassium phosphate, dipotassium phosphate, monosodium phosphate and disodium phosphate. In the present invention, it is preferable to use a mixture  
20 of phosphoric acid or a derivative thereof and a salt thereof, or a mixture of a few kinds of phosphates or salts of the derivatives.

The concentration of phosphoric acid, or the derivative or salt thereof in the medium must be at  
25 least 1 mM from the viewpoint of the effect, and it is desirable that the concentration be in a range of preferably 1 mM to 1 M, more preferably 5 mM to 500

mM, particularly preferably 100 mM to 500 mM.

The medium used in the present invention is not particularly limited so far as bacteria of the genus *Corynebacterium* can grow therein, and a medium  
5 containing a carbon source, a nitrogen source, metal minerals, vitamins, etc. in addition to the above-described saccharide and phosphoric acid or the derivative or salt thereof may be used.

Examples of other carbon sources than the  
10 saccharide include organic acid salts such as acetates.

Examples of the nitrogen source include ammonia, inorganic and organic ammonium salts such as ammonium chloride, ammonium sulfate, ammonium nitrate,  
15 ammonium carbonate, ammonium phosphate and ammonium acetate, and nitrogen-containing organic substances such as urea, peptone, meat extracts, yeast extracts and hydrolyzates of casein, and amino acids such as glycine, glutamic acid, alanine and methionine.

20 Examples of the metal minerals include sodium chloride, ferrous sulfate, magnesium sulfate, manganese sulfate, zinc sulfate and calcium carbonate. These metal minerals may be used either singly or in any combination thereof as needed.

25 The culture is conducted by suitably adjusting pH and temperature so as to become conditions under which the microorganisms can sufficiently grow.

However, it is generally preferred that the culture be conducted for 12 to 96 hours at a pH of 5 to 8 and a temperature of 25 to 40°C.

As a culturing method, a resting-bacterial  
5 reaction and an immobilized-bacterial reaction may also be used in addition to shaking culture and culture by a fermentor.

The collection of G-1-P produced and accumulated in the medium may be conducted by, for example,  
10 separating and removing the bacteria used and combining centrifugation, ultrafiltration, ion exchanging, reverse osmosis, electrodialysis, salting out, crystallization etc. with one another in accordance with a publicly known method.

15 According to the process of the present invention, a high concentration of G-1-P can be thus produced directly in the medium, and so a mass of G-1-P can be produced without conducting any complicated steps compared with the enzymatic process  
20 using an enzyme. As demonstrated in Examples, which will be described subsequently, such an effect is scarcely recognized in the MDPase derived from bacteria of the genus *Bacillus* (Japanese Patent Application Laid-Open No. 14580/1998) in which the  
25 MDPase is contained in the bacteria like the bacteria of the genus *Corynebacterium*, and the specific activity thereof is 4.2 U/mg and comparable with

MDPase derived from the bacteria of the genus  
*Corynebacterium* the specific activity of which is 5.3  
U/mg (J. Carbohydrate Chem., 14, 1017-1028 (1995)),  
and is a characteristic effect of the bacteria of the  
5 genus *Corynebacterium* .

<Determination of G-1-P>

The determination of G-1-P was conducted in  
accordance with the Weinhausle's method (Enzyme  
Microb. Technol., 17, 140-146 (1995)) with the method  
10 modified.

More specifically, an enzymatic reaction  
solution (100  $\mu$ L, containing 100 mM Tris-acetate  
buffer (pH 6.8), 2 mM EDTA, 10 mM magnesium sulfate,  
2 mM NAD, 10  $\mu$ M glucose-1,6-diphosphate, 1.2 U/mL  
15 phosphoglucomutase (derived from rabbit muscle,  
product of Roche Diagnostic Co.) and 1.2 U/mL  
glucose-6-phosphate dehydrogenase (derived from  
*Leuconostoc mesenteroides*, product of Roche  
Diagnostic Co.)) was added to a sample (100  $\mu$ L)  
20 suitably diluted on a 96-well microplate, and the  
mixture was incubated at 37°C for 30 minutes to  
measure an absorbance at 340 nm.

Example 1:

One platinum loop of *Corynebacterium callunae*  
25 IFO 15359, *Bacillus subtilis* IFO 3037, *Bacillus*  
*subtilis* IFO 1372 or *Bacillus licheniformis* JGM 2505  
was inoculated into a medium (containing 3.5% soluble

starch, 3.0% Lablemco powder (product of OXOID Co.),  
0.05% magnesium sulfate, 0.04% monopotassium  
phosphate and 0.1% disodium phosphate) to conduct  
shaking culture overnight at 30°C as species culture.

5        Each (1%) of the species bacteria cultured above  
was inoculated on a medium prepared by adding a  
potassium phosphate buffer (pH 7.0) to 3.5% soluble  
starch, 3.0% Lablemco powder (product of OXOID Co.),  
0.05% magnesium sulfate so as to give a phosphate  
10       concentration of 10 mM, 20 mM or 40 mM to conduct  
culture at 30°C for 5 days as principal culture. The  
concentration of G-1-P in a supernatant thus obtained  
was determined. The results are shown in Table 1.

Table 1

15                      Productivity of G-1-P (g/L)

Concentration of phosphate	10 mM	20 mM	40 mM
<i>C. callunae</i> IFO 15359	0.15	0.41	0.58
<i>B. subtilis</i> IFO 3037	<0.01	<0.05	<0.01
<i>B. subtilis</i> IFO 1372	0.03	<0.01	<0.01
<i>B. licheniformis</i> JGM 2505	0.07	0.01	0.01

Example 2:

One platinum loop of *Corynebacterium callunae*  
IFO 15359, *Corynebacterium glutamicum* JCM 1321,  
20       *Corynebacterium vitaeruminis* JCM 1323, *Bacillus*  
*subtilis* IFO 3037, *Bacillus subtilis* IFO 1372 or  
*Bacillus licheniformis* JGM 2505 was inoculated into a



medium (containing 3.5% soluble starch, 3.0% Lablemco powder (product of OXOID Co.), 0.05% magnesium sulfate, 0.04% monopotassium phosphate and 0.1% disodium phosphate) to conduct shaking culture  
5 overnight at 30°C as species culture.

The species bacteria cultured above were respectively collected, and each of them was inoculated on a medium prepared by adding a potassium phosphate buffer (pH 7.0) to 0.67% yeast nitrogen  
10 base (product of Difco Co.) and 10% dextrin (derived from potato, product of SIGMA Co.) so as to give a phosphate concentration of 100 mM, 200 mM, 400 mM or 500 mM in such a manner that OD<sub>600 nm</sub> is 10, thereby conducting culture at 30°C for 5 days as principal  
15 culture. The concentration of G-1-P in a supernatant thus obtained was determined. The results are shown in Table 2.

Table 2  
Productivity of G-1-P (g/L)

Concentration of phosphate	100 mM	200 mM	400 mM	500 mM
<i>C. callunae</i> IFO 15359	2.1	5.1	12.7	12.7
<i>C. glutamicum</i> JCM 1321	-	-	11.5	-
<i>C. vitaeruminis</i> JCM 1323	-	-	13.0	-
<i>B. subtilis</i> IFO 3037	0.01	0.02	-	0.04
<i>B. subtilis</i> IFO 1372	0.02	0.03	-	0.02
<i>B. licheniformis</i> JGM 2505	0.01	0.01	-	0.01

As a result of the above, G-1-P was able to be produced with good efficiency depending on concentration by using the bacteria of the genus *Corynebacterium* and adjusting the concentration of the phosphate.

According to the process of the present invention, as described above, a mass of G-1-P can be provided without conducting complicated steps such as a step of extracting an enzyme from plants or bacteria and preparation of an immobilized enzyme, which are required of the enzymatic process.